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Molecular Reappraisal of Relationships Between *Crataegus* and *Mespilus* (Rosaceae, Pyreae)—Two Genera or One?

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ABSTRACT. *Mespilus* and *Crataegus* are sister genera in Rosaceae tribe Pyreae. *Mespilus* has been seen to comprise not only the medlar, *Mespilus germanica*, of western Eurasia but also the Arkansas, U.S.A. endemic, *Mespilus canescens*. *Crataegus*, on the other hand, consists of 140–200 species found throughout the northern hemisphere. Diagnoses of these two genera rely on morphological features of leaves, flowers and fruits. However, character states supposed to be diagnostic of *Mespilus* occur in species of *Crataegus*. We used two nuclear (ribosomal ITS and *LEAFY* intron2) and four intergenic chloroplast DNA regions (*trnS-trnG, psbA-trnH, trnH-rpl2*, and *rpl20-rps12*) to estimate the phylogeny of *Mespilus* and *Crataegus*. Maximum parsimony, maximum likelihood, and Bayesian analyses all corroborate the sister group relationship between *Crataegus* and *Mespilus*, and *Crataegus brachyacantha* sister to the rest of *Crataegus*. However, incongruence between chloroplast and nuclear data supports the hypothesis of a hybrid origin for *Mespilus canescens*, with *Crataegus brachyacantha* or its ancestor as the maternal parent. Accordingly, we (1) restrict *Crataegus* section *Brevispinae* to *Crataegus brachyacantha* (2) distinguish the Arkansas endemic as a nothospecies; (3) describe a new section and a new nothosection within *Crataegus* to contain the former species of *Mespilus* and *Crataemespilus*; and (4) make two new combinations under *Crataegus*.

KEYWORDS: *Crataegus*, generic delimitation, hybrid origin, *Mespilus*, molecular data, phylogeny.

Crataegus and *Mespilus* have a complicated taxonomic history. In brief, the modern concepts of *Crataegus* and *Mespilus* originated with Medikus (1793), and are based on the way in which the pyrenes are covered in *Mespilus* but exposed in the fruits of *Crataegus*. According to Medikus, *Mespilus* comprised a single species, the medlar, *M. germanica* L. (Medikus 1793). *Crataegus*, on the other hand, consisted of 12 hawthorn species and one species of what is now recognized as the genus *Pyracantha* M. Roem. Lindley (1822) maintained Medikus' concept of the two genera, but reversed his distinction between them by suggesting that ''in *Mespilus* the top of the cells is absolutely naked; and this is one of the distinctions between it and *Crataegus*,'' perhaps confusing the openness of the free portion of the hypanthium in the medlar fruit with the lack of any tissue covering the pyrenes. Despite alternative interpretations of these genera by others (see Table 1 in Robertson et al. 1991), this concept of *Crataegus* and a monotypic *Mespilus* espoused by Medikus and Lindley was maintained by Candolle (1825), Decaisne (1874), and Koehne (1890), and is the concept that has been in use throughout the twentieth century.

More recently, the similarities and differences between *Crataegus* and *Mespilus* have been explored in the context, on the one hand, of expanding *Mespilus* to include a North American entity endemic to Arkansas, *M. canescens* J. B. Phipps, and on the other, of renewed interest as a result of data from molecular systematic studies in generic limits within Rosaceae tribe Pyreae Baill. (formerly treated as subfamily Maloideae). Several molecular phylogenies have demonstrated a sister-group relationship between *Crataegus* and *Mespilus* (Campbell et al. 1995; Evans et al. 2000; Evans and Campbell 2002; Campbell et al. in press). In many of these analyses, *Amelanchier* Medik. and its related genera *Peraphyllum* Nutt. ex Torr. and Gray and *Malacomeles* (Decne.) Engl. have been shown to be sister to the *Crataegus-Mespilus* clade. There are more morphological differences, however, between the *Amelanchier* group and the *Crataegus-Mespilus* clade than there are between *Mespilus* and *Crataegus*. On the one hand, vegetative growth on fertile short shoots is sylleptic in *Amelanchier*, distinguishing it from most of the other genera in Pyreae that have proleptic sympodial development of lateral short shoots. On the other hand, *Crataegus* and *Mespilus* are distinguished from the *Amelanchier* group and most other Pyreae by (1) lateral short shoots modified as thorns; (2) collateral ovules that become superposed by the time of anthesis so that typically only the lower one is fertilized, (3) abundant endosperm in the mature seed (Aldasoro et al. 2005), and (4) a polypyrenous drupe (rather than a berry or ''pome'') that develops from the hypanthial ovary. Variation within *Crataegus* in leaf margination and venation, number of flowers per

inflorescence, and in stamen number per flower, encompasses most or all of the states exhibited in *Mespilus*. While *M. germanica* has been shown to be a diploid, like many species of *Crataegus, M. canescens* proves to be triploid and largely sterile (Talent and Dickinson 2005; Dickinson unpubl. data). Phipps et al. (1991) argued that their phenetic analyses of isozyme data collected from both species of *Mespilus*, several species of *Crataegus*, and a number of outgroup Pyreae genera supported the naturalness of *Mespilus* as a genus. Because of concern about the failure of the Konecny Grove population to recruit new individuals, McCue et al. (2001) used four RAPD primers to identify unique genotypes for ex situ conservation (seed set by *M. canescens* grown at the Dale Bumpers Small Farms Research Center, Booneville, Arkansas, from Konecny Grove seedlings is extremely poor; two seeds, in 61 pyrenes from 13 fruits). Using 10 consistently amplified bands, comparison of RAPD phenotypes in the 25 *M. canescens* individuals in Konecny Grove with the phenotype of a single individual of *M. germanica* (McCue et al. 2001) demonstrated, upon reanalysis of these data (not shown; these results differ slightly from those reported by McCue et al.), the presence of 11 unique RAPD phenotypes in the *M. canescens* individuals (one to eight individuals per phenotype), none of which had any of the 10 bands in common with the *M. germanica* individual. More recently, Verbilaitė et al. (2006) demonstrated the similarity of DNA sequences from *M. germanica* and *M. canescens* to those of some *Crataegus* species for the *trnL-trnF* region of the chloroplast genome. These are the only molecular data that have been adduced to date. The present study seeks to resolve the relationship between these two genera using DNA sequence data from both the chloroplast and nuclear genomes.

This paper is part of a larger project on *Crataegus* systematics and evolution that has the following objectives: (1) to evaluate the support for *Mespilus* and *Crataegus* as distinct genera; (2) to unravel the origin and relationships of *M. canescens* with other *Mespilus* and *Crataegus* taxa; (3) to discover the intrageneric taxonomic structure within *Crataegus* and find out to what extent the the existing subgeneric classification represents distinct clades; (4) to infer the phylogenetic and biogeographic relationships between diploid and polyploid *Crataegus* entities and; (5) to establish what species concept best reflects the biology and evolutionary history of the North American black-fruited hawthorns (sections *Brevispinae* Beadle ex C.K.Schneid. and *Douglasianae* Loud.).

This paper focuses on the first two of these objectives. We use a combination of nuclear and chloroplast sequences to infer the phylogeny of mainly diploid *Mespilus* and *Crataegus* species (Appendix 1). The commonly used nuclear ribosomal internal transcribed spacers (ITS) and the second intron of the floral homeotic gene, *LEAFY* were selected to represent the nuclear genome. *LEAFY* appears to be single copy in most angiosperms (Frohlich and Meyerowitz 1997), but two orthologues have been reported in *Malus* species (Wada et al. 2002). The second intron has been informative in some previous phylogenetic studies (Archambault and Bruneau 2001; Grob et al. 2004) and it provided twice as many informative characters as the ITS and 10 times more than the cpDNA data among genera of the Rosaceae (Oh and Potter 2003, 2005). In addition to the nuclear sequences, four non-coding chloroplast regions *trnS-trnG, psbA-trnH, trnH-rpl2*, and *rpl20-rps12*, adjacent to the junction of the large single copy (LSC) and inverted repeat (IR) were used. These regions have been demonstrated informative for inferring phylogenies at both inter- and intraspecific levels (Goulding et al. 1996; Xu et al. 2000; Vaillancourt and Jackson 2000). Together, they provide an independent plastid phylogeny that can be compared with the nuclear trees.

Materials and Methods

Taxon Sampling. Plant material was either collected in the field or from botanical gardens (Appendix 1). Voucher specimens are deposited in the Green Plant Herbarium of the Royal Ontario Museum (TRT) unless noted otherwise in Appendix 1. Although every effort was made to include only diploid taxa of the genus *Crataegus*, two factors led to the inclusion of some polyploids in the samples studied here. First, we sought to represent as many sections of the genus as possible. In some cases where a section is monotypic (*Parvifolieae* Loudon, *Cordatae* Beadle ex C.K.Schneid.), it was necessary to use a polyploid entity (Appendix 1; Talent and Dickinson 2005). Second, sampling for this project took place before or concurrently with sampling for a parallel study of variation in nuclear DNA content (Talent and Dickinson 2005) so that, in some instances, we discovered that species we sampled vary in ploidy level (e.g. *C. laevigata* Poir., *C. monogyna* Jacq.; Appendix 1). Other species, such as *C. crus-galli* L. and *C. suksdorfii* (Sarg.) Kruschke (Appendix 1), we knew varied in ploidy level, but we were interested in including them in our study. A total of 31 *Crataegus* and two *Mespilus* species were included with, in most cases, a minimum of two individuals representing each species. In three cases, only a single individual was available to represent a section or series (sections *Mexicanae* Loud. and *Lacrimatae* (J.B.Phipps) J.B.Phipps, and series *Triflorae* (Beadle) Rehder in section *Coccineae* Loud.; Appendix 1). In some other cases where more than one species was available to represent a section or series, some species were represented by a single individual (Appendix 1). One individual was included in the sample on the supposition that it represented *C. cuneata* Siebold. and Zucc. (section *Cuneatae* Rehder ex Schneider), but comparison with the image of the type specimen of this species demonstrated that this is not the case and this accession is listed under *incertae sedis* (Appendix 1).

Species of *Amelanchier, Malus* and *Aronia* were used as outgroups because they have been shown to be divergent to varying degrees from *Crataegus* and *Mespilus* (Campbell et al., in press).

Morphological Data. Data on vegetative and reproductive morphology (Appendix 2) are based on field observations and herbarium specimens, and on data in Robertson et al. (1992), and Phipps et al. (2003). Secondary venation of short shoot leaves was visualized on x-ray negatives prepared using a Hewlett-Packard Faxitron x-ray system and Kodak Industrex film.

DNA Extraction, PCR, and Sequencing. Total genomic DNA was extracted from leaves that were either frozen on dry ice and stored at −80°C or dried on silica gel and stored at room temperature. Frozen samples were extracted using the modified CTAB procedure of Doyle and Doyle (1987), while dried leaves were extracted using the method of Tsumura et al. (1995) modified to a small scale. The nuclear ribosomal region encompassing ITS-1, 5.8S rRNA and ITS2 spacer was amplified using primers ITS4 and ITS5 (White et al. 1990). The second intron of *LEAFY* was amplified using primers LFY1 and LFY2 designed on the 2′ and 3′ exon (Oh and Potter 2003). Four chloroplast intergenic spacer regions *psbA-trnH* (Sang et al. 1997), *rpl20-rps12* and *trnG-trnS* (Hamilton 1999), and *trnH-rpl2* (Vaillancourt and Jackson 2000) were amplified using the published primers.

Each 25 µl PCR reaction contained 5 pmol each of 5′ and 3′ primer, 0.2 mM dNTP, 1 unit of Taq DNA polymerase (Fermentas), 2.5 mM $MgCl_2$, and 2.5 µl 10× PCR buffer. DMSO was added to a final 10% in both ITS and *LEAFY* amplifications to increase the specificity of the PCR fragments and the intensity of the sequence peak profiles. All amplifications were carried out using a T1 Thermocycler (Whatman Biometra, Göttingen, Germany). PCR cycles involved an initial denaturing step at 94°C for 3 min, then 35 cycles of 94°C for one min, 50–56°C for 50 s, and 72°C for 2 min. An additional extension was performed at 72°C for five min, then cooled to 4°C. PCR products were checked on 1% agarose gels. All chloroplast amplicons were sequenced directly after purification with MinElute purification columns (Qiagen Inc., Valencia, California). Purified PCR products of ITS and *LEAFY* were cloned following the protocol of Qiagen's pDrive Vector System and 3–5 clones per sample were sequenced using Perkin-Elmer BigDye terminator kits on ABI Model 3100 automated sequencer (PE Applied Biosystems, Inc., Foster City, California).

Sequence Editing, Alignment, and Phylogenetic Analyses. Multiple alignments of sequences were first obtained using the ClustalX program (Thompson et al. 1994) and then manually edited in Sequence Alignment Editor (Rambaut 2002). Gaps within the sequence data were treated as missing. However, the parsimony informative gaps, i.e. gaps shared by at least two ingroup species as determined by visual inspection of the alignment, were coded as either binary (presence or absence of indels) or multistate characters (depended on the length of indels) and appended to the sequence matrixes for phylogenetic analyses (Guillon 2004). Representative sequences for each region for each species were deposited in GenBank (Appendix 3; accessions EF127007–127228).

Phylogenetic analyses were conducted using PAUP*4.0b (Swofford 2002) for maximum parsimony (MP) and maximum likelihood (ML), and MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001) for Bayesian inference (BI). Nuclear and chloroplast data were analysed both separately and jointly with the three methods. In order to obtain phylogenies based on a complete dataset, taxa in conflicting positions in the nuclear and chloroplast trees were removed in the combined analysis. Heuristic parsimony searches were performed using equally-weighted characters, tree-bisection-reconnection (TBR) branch swapping, random addition of sequence (1000 replicates), and with no limit to the number of trees saved. Character changes were interpreted with the ACCTRAN optimization. Branch support was assessed by bootstrap (BS) analyses (Felsenstein 1985) with full heuristic searches, 500 replicates using simple taxon addition and TBR swapping, MULTtrees option off.

In order to reduce computational time, one individual per species was included in the ML analyses of nuclear, chloroplast, and the combined data. The substitution models for ML and Bayesian analyses were obtained using Modeltest (version 3.06, Posada and Crandall 1998) with both Hierarchical Likelihood Ratio Tests (hLRTs) and Akaike Information Criterion (AIC) methods. Maximum likelihood analysis of the combined nuclear data was conducted with Transitional (TIM) model (parameters: base frequencies A = 0.1917, C = 0.3429, G = 0.3013, T = 0.1641, proportion of invariable sites (I) 0.5183, gamma 1.1819,Ti/Tv 1.463, 6 rate parameters and molecular clock not enforced). Analysis of the chloroplast data was conducted with the General Time Reversible (GTR) model (parameters: base frequencies A = 0.3538, C = 0.1332, G = 0.1456, T = 0.6536, proportion of invariable sites (I) 0.6536, gamma 0.4233, Ti/Tv 0.622, 6 rate parameters and molecular clock not enforced). The smaller gamma value obtained in the chloroplast dataset compared with that in the nuclear data indicated a more substantial heterogeneity of rate substitution across the chloroplast nucleotides. Analysis of the combined nuclear and chloroplast data was conducted with the Transversional (TVM) model (parameters: base frequencies A = 0.3095, C = 0.1852, G = 0.1862, T = 0.3191, proportion of invariable sites (I) 0.5093, gamma 0.567, Ti/Tv 1.6414, 6 rate parameters and molecular clock not enforced).

Bayesian inference was initiated from a random starting tree and the program was set to run four Markov chain Monte Carlo (MCMC) iterations for 1,000,000 generations with trees sampled every 100th generations. The likelihood scores, trees, and other sample points generated prior to 136,100 and 55,700 generations respectively for nuclear and chloroplast data were discarded because they do not provide accurate parameter estimates. The remaining trees were saved and imported into PAUP* for constructing the majority rule consensus trees. Posterior probability for each clade was obtained to evaluate branch support in the resulting trees.

Alternative Topologies. We used the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) as implemented in PAUP* (Swofford 2002) to compare the best ML trees recovered respectively from the nuclear and chloroplast data with the constraint trees constructed in MacClade (Maddison and Maddison 1992). To test the two genera hypothesis, *Crataegus* and *Mespilus* taxa were constrained into two monophyletic groups and the trees were loaded as backbone into PAUP*. Heuristic searches were conducted using the same ML parameters outlined above to find the shortest trees compatible with the constraint. The likelihood score of the constrained tree was then compared with the score of the best ML tree using the one tailed non-parametric SH tests.

RESULTS

Sequences. Our data conform to the generally lower GC content in the chloroplast sequences than that in the nuclear sequences (Table 1). For the nuclear sequences, intraspecific polymorphism was no more than 0.01% among clones of our examined taxa including the triploid *C. uniflora* and *M. canescens*, and tetraploid *C. phaenopyrum*.

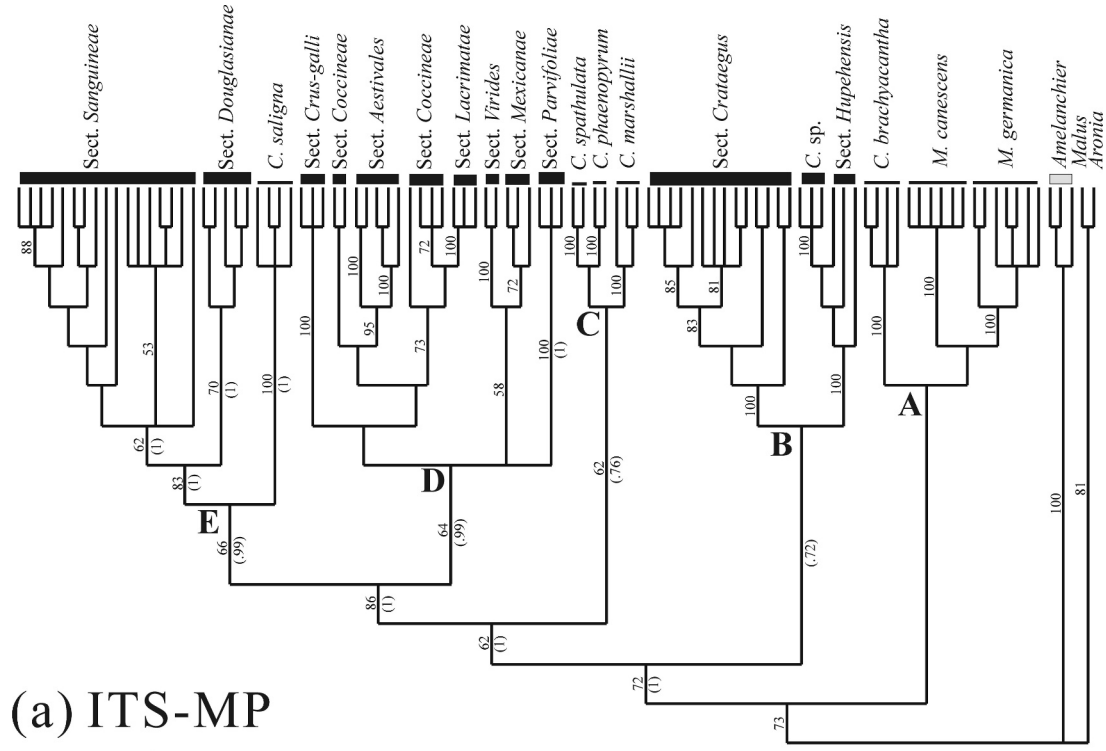
TABLE 1. Comparison of sequence variation in *Crataegus, Mespilus,* and outgroups for the two nuclear and four chloroplast regions. PI = parsimony informative; MPT = most parsimonious tree; C.I. = consistency index; R.I. = retention index.

	Nuclear (NR) sequences			Plastid (CP) sequences						
	ITS	*leafy*	combined nr	*trnG-trnS*	*psbA-trnH*	*trnH-rpl2*	*rpl20-rps12*	combined cp	NR + CP
Number of sequences	156	156	156	82	82	82	82	82	77
Number of characters	671	696	1367	719	344	287	736	2085	3452
GC content (%)	66.3	43.25	64.42	30	27	31	39	30.2	37
Number of variable characters	203	282	539	70	41	37	54	211	726
Number of PI characters with outgroup	168	205	436	54	21	18	34	125	552
Number of PI characters without outgroup	151	93	243	41	18	13	33	105	272
Number of observed PI indels	13	8	21	8	7	0	4	19	40
Divergence range within ingroup (%)	0.31–8.11	0.23–8.56	0.34–6.67	0–2.71	0–3.01	0–2.79	0–1.94	0–2.56	0–3.45
Divergence range between ingroup and outgroup (%)	4.91–9.64	1.13–34.29	3.86–21.91	1.99–4.33	1.53–3.95	1.73–4.86	1.12–2.63	2.21–5.85	2.76–11.54
Divergence within genus *Crataegus* (%)	0.3–9.2	0.6–9.1	–	–	–	–	–	0.44–3.32	–
Divergence within genus *Mespilus* (%)	6.25	1.7–2.2	–	–	–	–	–	1.45	–
Number of MPTs	2,761	27,684	970	1,366	31,500	30,300	30,627	18,432	29,113
Tree length	509	411	917	98	89	322	85	335	1141
C.I.	0.73	0.82	0.74	0.93	0.64	0.6	0.84	0.75	0.81
R.I.	0.91	0.94	0.89	0.97	0.84	0.78	0.92	0.87	0.89

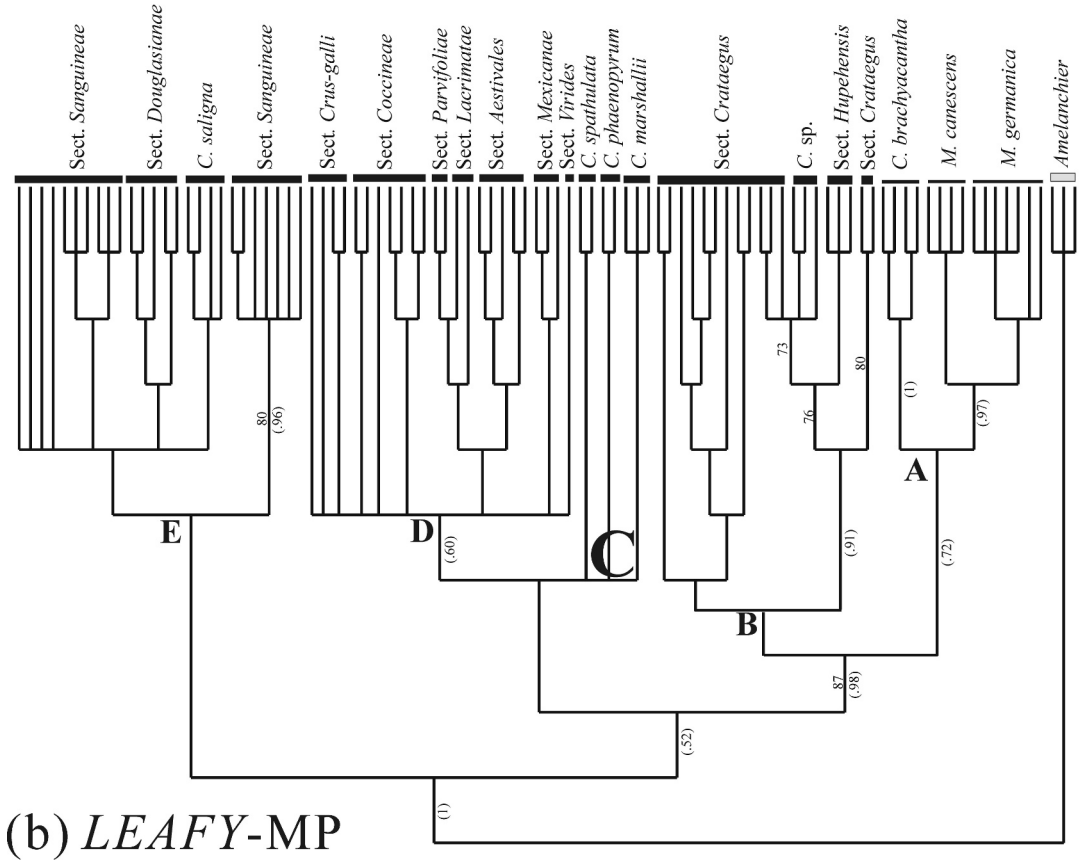
Size variation was observed in both nuclear regions (Table 1). In *LEAFY*, divergence between ingroup and outgroup taxa (*Malus* and *Aronia*) was as much as 35%, which was about three-fold higher than in the ITS region (Table 1). Because of the alignment difficulties with the divergent sequences, *Malus* and *Aronia* were removed in the phylogenetic analyses. The spacer regions between the chloroplast genes, like those in the ITS and *LEAFY* intron, showed noticeable length variation across sequences of our studied taxa, and gave a total of 19 parsimony informative indels in the combined data matrix (Table 1). Most of the indels were conserved in sequences and can be easily aligned except an AT-rich indel which was 245 bp long in the *trnH-rpl2* region. Taxa showed remarkable variation in the length of this indel caused by irregular AT insertion; therefore, this region was excluded in phylogenetic analyses.

Nuclear Phylogeny. In all analyses, *Amelanchier* was shown to be less divergent from the ingroup taxa than were *Malus* and *Aronia*. Heuristic parsimony searches of the ITS data alone yielded 2761 equally parsimonious trees. Within the ingroup, *Mespilus* taxa were monophyletic, but this was not the case for the *Crataegus* taxa because of *Mespilus* (Fig. 1a). *Crataegus brachyacantha* (section *Brevispinae*) was associated with the two *Mespilus* species and was distinct from the rest of the genus (clade A). This relationship was supported additionally by two indels detected in the alignment. The remaining *Crataegus* taxa are divided into four clades labeled as B, C, D, and E with moderate bootstrap or Bayesian support (Fig. 1a). Clade B contains members of the Eurasian sections *Crataegus* and *Hupehensis*. Clade C is a small group of three North American taxa: *C. marshallii* (sect. *Crataegus*), *C. phaenopyrum* (sect. *Cordatae*), and *C. spathulata* (sect. *Microcarpae*). Clade D contains members of section *Coccineae, Crus-galli, Virides, Mexicanae*, and *Aestivales* exclusively from eastern North America, and this whole group was sister to clade E that contains members of sections *Sanguineae* and *Douglasianae,* and *C. saligna* (sect. *Brevispinae*). Over all of the ingroup branches, the unrooted tree of the ITS data showed a maximum of 27 changes compared with 40 changes on the branch leading to the outgroup taxa.

In contrast, with the *LEAFY* data, about 205 changes were accumulated along the branch leading from *Malus* and *Aronia* to *Amelanchier* and the ingroup (branch lengths in this area of the tree < 15 changes. Thus, over 27,000 parsimony trees were produced when *Malus* and *Aronia* were included as outgroup. We conclude that the extremely long branch of *Malus* and *Aronia* in the



(a) ITS-MP



(b) *LEAFY*-MP

LEAFY data could have distorted the topology of clades with relatively short branches, and resulted in an inaccurate phylogeny. In order to alleviate this rooting problem, further analyses of the *LEAFY* data used *Amelanchier* as the only outgroup.

Without *Malus* and *Aronia*, the *LEAFY* data yielded a total of 5053 parsimony trees. The strict consensus tree (Fig. 1b) divided the ingroup taxa into three main clades: {A, B}, {C, D}, and E. As in the ITS data (Fig. 1a), *C. brachyacantha* was allied with the *Mespilus* species (clade A), but with poor support (< 50% BS). This clade was strongly associated with the Eurasian taxa of *Crataegus* (clade B; 87%BS, 98%BI). The three monotypic groups (sections *Cordatae* and *Microcarpae*, and series *Apiifoliae*, in section *Crataegus*) which constituted clade C in the ITS data (Fig. 1a) were unresolved in *LEAFY* and were found in a polytomy together with the other eastern North American taxa (clade D; Fig. 1b). A similar pattern was also found in the ML tree (data not shown), as well as in the Bayesian results where the eastern North American taxa were resolved as a polytomy (data not shown).

Because there was no strongly supported conflict between the topologies inferred from the ITS (Fig. 1a) and *LEAFY* (Fig. 1b) data, the two datasets were combined to increase robustness and phylogenetic resolution. Analysis of the combined nuclear data resulted in 970 equally parsimonious trees. The strict consensus tree (Fig. 2) demonstrated the monophyly of *C. brachyacantha* and the *Mespilus* species (clade A; Figs. 1a, b), and this clade was found to be closely related to the Eurasian species (clade B), as shown in the *LEAFY* data (Fig. 1b). However, this association was weakly supported in the bootstrap analysis (BS < 50%). Clades D and E were well supported as sister groups as shown in the ITS data (Fig. 1a), and clade C was shown adjacent to clades {D, E}.

Chloroplast Phylogeny. Maximum parsimony analyses of individual chloroplast region each recovered over 30,000 equally parsimonious trees with only a few resolved clades nested in widely unresolved topologies. Because the entire chloroplast genome is considered as one linkage group, individual regions are expected to exhibit the same phylogenetic pattern (Doyle 1992). We combined all sequences to obtain greater phylogenetic resolution.

Heuristic parsimony analyses of combined chloroplast data produced 18,432 trees. Clades A, B, D, and E, as found in nuclear data (Fig. 2), were recovered in the chloroplast data (Fig. 3). However, apparent conflicts were detected in the relationships between *C. brachyacantha* and the *Mespilus* taxa within clade A, and in the position of *C. marshallii, C. phaenopyrum*, and *C. spathulata* of clade C in the chloroplast and nuclear trees. Such incongruences were also supported by the ML (Fig. 3) and Bayesian results (data not shown). None of the analyses of the chloroplast data recovered *M. canescens* and *M. germanica* as a monophyletic group (Fig. 3). Interestingly, *Mespilus* was recovered as paraphyletic, with *M. canescens* more closely related with *C. brachyacantha* than with *M. germanica* (BS ≥ 81 and BI ≥ 97). This association coincided with 16 site changes and 5 diagnostic indels shared between the two taxa as detected in the alignment. Another major conflict was found in clade C in which the association of *C. phaenopyrum, C. marshallii,* and *C. spathulata* collapsed and these taxa were dispersed within the eastern and western North American taxa (clades D and E).

Maximum Likelihood Analyses and Tests of Alternative Phylogenetic Hypotheses. For the combined nuclear data, ML analysis using TIM+G+I model ($r_{AC} = 1.00$, $r_{AG} = 1.77$, $r_{AT} = 0.64$, $r_{CG} = 0.64$, $r_{CT} = 2.48$, $\alpha = 1.18$, $p_{inv} = 0.52$) recovered a single tree (Fig. 4a) with –lnL = 5,894.64. Topology was found similar to the MP (Fig. 2) and Bayesian (data not shown) results. Results of the Shimodaira–Hasegawa test based on nuclear data failed to reject the hypothesis that *Crataegus* and *Mespilus* are two separate monophyletic groups ($P = 0.145$). The difference in likelihood scores between the best ML tree and an ML tree constrained to fit the hypothesis was $-5{,}894.64 - (-5{,}922.35) = 27.71$. Hence, the result of the SH test on the nuclear sequence data is consistent with the traditional treatment of *Crataegus* and *Mespilus* as two distinct genera.

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FIG. 1. Strict consensus trees, from maximum parsimony (MP) analyses of (a) ITS1-5.8S-ITS2 (2761 trees) and (b) *leafy* second intron sequence data (27684 trees). Nodes with bootstrap (BS; above branches) and Bayesian posterior probability (BI; below branches) values > 50% are indicated. In (a) *Amelanchier, Malus,* and *Aronia* are used as outgroups, while in (b) *Amelanchier* is the only outgroup because of the extreme divergence in *Malus* and *Aronia* (details in text). Each branch represents a sequence obtained from at least three clones of an individual. Sectional affiliations of the *Crataegus* taxa (Phipps et al. 1990) are indicated by thick lines on the right, while species of *Mespilus* and three monotypic sections of *Crataegus* are indicated by thin lines. Major clades are labeled as A (*C. brachyacantha* and *Mespilus* species; B (taxa of sections *Crataegus* and *Hupehensis*); C (*C. marshallii, C. phaenopyrum*, and *C. spathulata*); D (taxa of eastern North American sections, and E (*C. saligna* and taxa of sections *Douglasianeae* and *Sanguineae*).

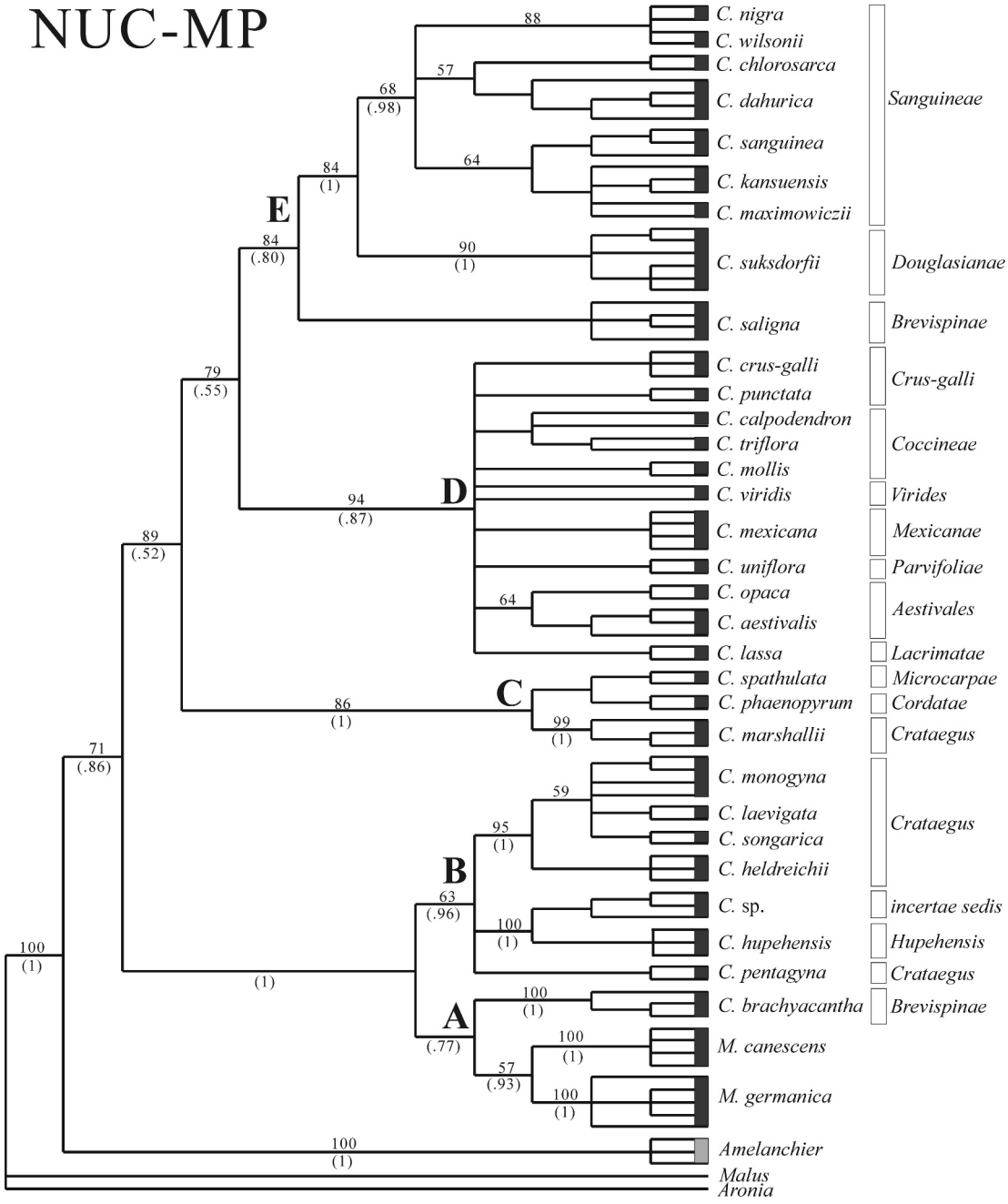

FIG. 2. Strict consensus of 790 maximum parsimony (MP) trees from the combined analysis of ITS and *leafy* second intron data. Nodes with bootstrap (BS; above branch) and posterior probability (BI; below branch) values > 50% are indicated. Species, sections, and genera (Phipps and Robertson 1990) are listed on the right. Labels of clades A–E as in Fig. 1.

Maximum likelihood analysis of chloroplast data using the GTR+G+I model (r_{AC} = 0.99, r_{AG} = 1.14, r_{AT} = 2.15, r_{CG} = 0.89, r_{CT} = 1.578, α = 0.42, p_{inv} = 0.65) recovered a single tree with –lnL = 4,228.18 (Fig. 4b). This tree supported the topology observed in the parsimony (Fig. 3) and Bayesian (data not shown) analyses except that the Eurasian taxa, *M. germanica*, and *M. canescens*-*C. brachyacantha* (i.e. clade A1, A2, and B in Fig. 4b) were resolved in a polytomy. Results of the SH test based on chloroplast data led us to reject the hypothesis that *Crataegus* and *Mespilus* are two separate monophyletic groups ($P < 0.05$). The difference in likelihood scores between the best ML

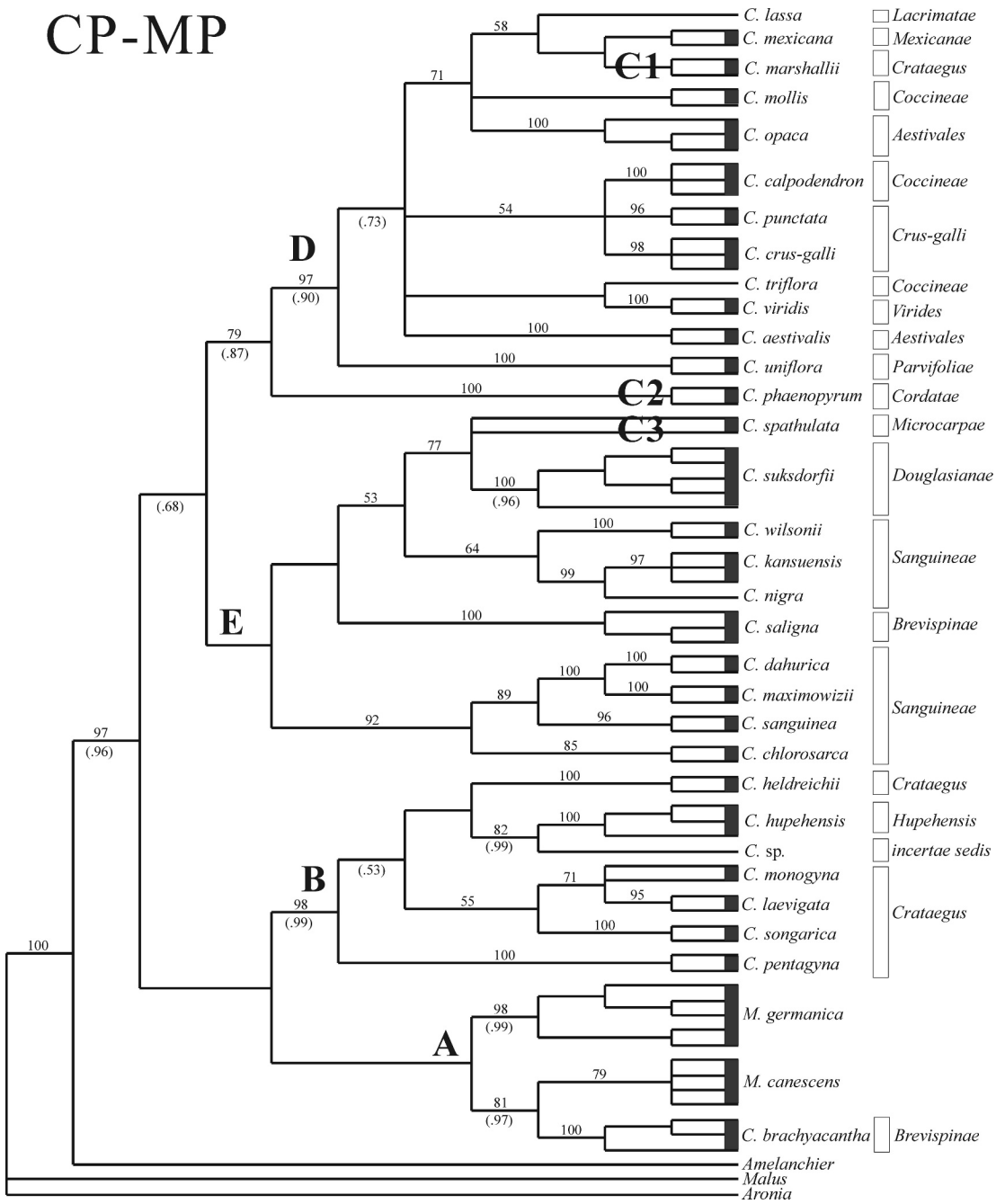

FIG. 3. Strict consensus of 18,432 equally parsimonious trees from the maximum parsimony (MP) analysis of the combined *trnG-trnS, psbA-trnH, trnH-rpl2*, and *rps20-rpl12* data. Nodes with bootstrap (BS; above branch) and posterior probability (BI; below branch) values > 50% are indicated. Species, sections, and genera (Phipps and Robertson 1990) are listed on the right. Labels of clade A–E can be referred to Figure 1.

tree and an ML tree constrained to fit the hypothesis was −4,228.18 − (−4,559.20) = 331.02. The SH test rejected the inclusion of *C. brachyacantha* within the *Crataegus* clade.

Combined Nuclear and Chloroplast Phylogeny. In order to test the two-genera hypothesis of *Crataegus* and *Mespilus* more thoroughly, we analyzed the combined nuclear and chloroplast data after removing the four taxa responsible for conflicting topologies (*M. canescens, C. marshallii, C. phaenopyrum,* and *C. spathulata*). Parsimony analyses generated 29,113 trees and four of the five

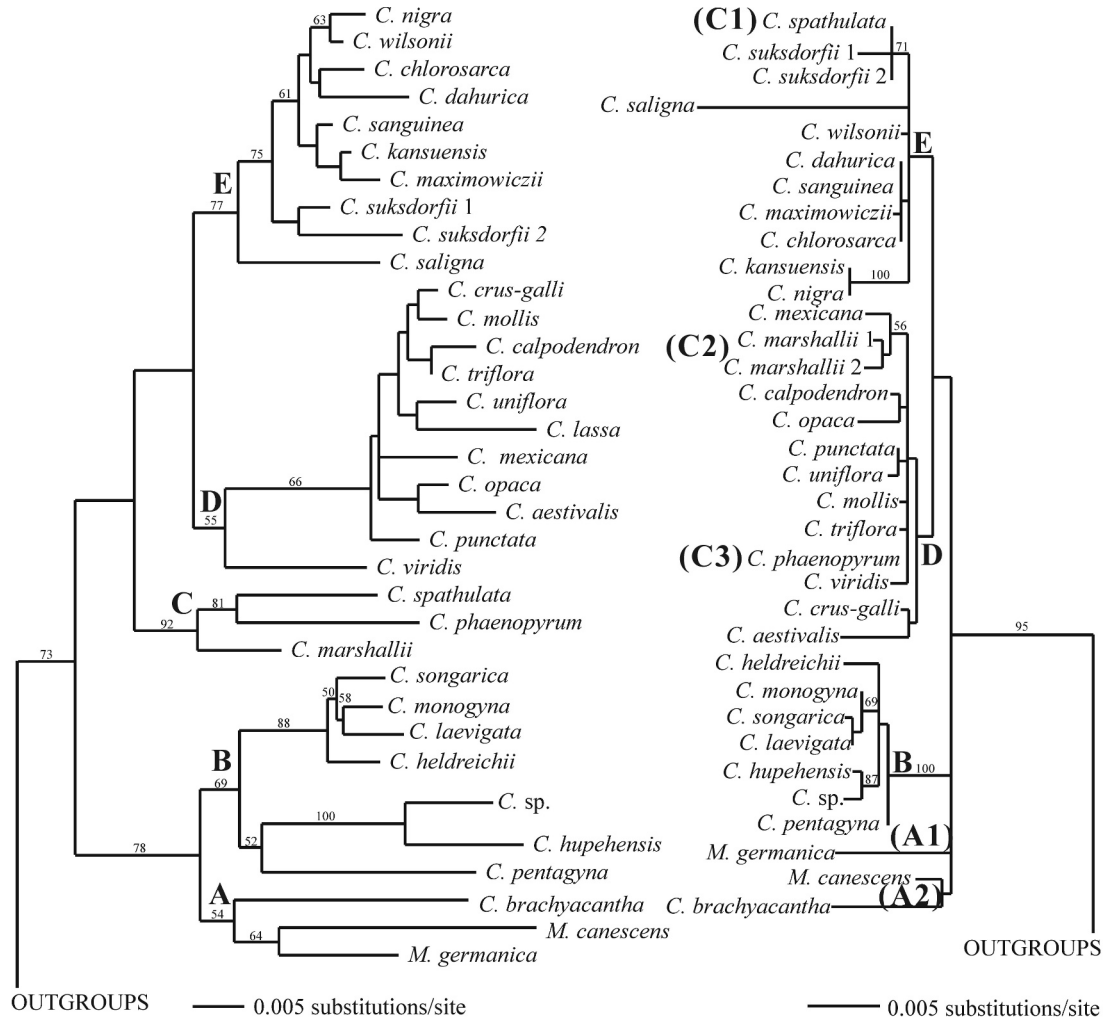

FIG. 4. The maximum likelihood (ML) trees of the combined nuclear (a) and chloroplast (b) data, generated by PAUP* using the TIM and GTR models, respectively. For the nuclear data, lnL: −5894.64, I = 0.52 and G = 1.18; while for the chloroplast data, lnL: −4228.18, I = 0.65 and G = 0.42. Nodes with bootstrap (BS) values > 50% are indicated above branch.

major clades (A, B, D, and E) obtained in the earlier analyses (Figs. 1–4) were recovered in the strict consensus tree (Fig. 5a). Bootstrap and posterior probability values were generally high among most clades (BS > 80% and BI > 97%) in this analysis. Only the association between *C. brachyacantha* and *M. germanica* was not strongly supported (BS = 57% and BI = 77%). The difference in likelihood scores between the strict consensus MP tree and constrained MP tree was −11,001.39 − (−10,968.21) = 33.19. The Shimodaira–Hasegawa test based on the combined data failed to reject the hypothesis that *Crataegus* and *Mespilus* are two separate monophyletic groups only when *M. canescens* was removed ($P = 0.096$).

Maximum likelihood analysis of the combined data using the TVM+G+I model ($r_{AC} = 1.06$, $r_{AG} = 2.15$, $r_{AT} = 2.89$, $r_{CG} = 1.14$, $r_{CT} = 2.15$, $\alpha = 0.57$, $p_{inv} = 0.51$) recovered a single tree with –lnL = 11,001.39 (Fig. 5b). In the ML tree, the association of *C. brachyacantha* and *M. germanica* (clade A) collapsed and *C. brachyacantha* was clearly sister to all *Crataegus* species.

DISCUSSION

Intergeneric Divergence of* LEAFY *Sequences. There are not many nuclear genes that have been used in the phylogeny of Pyreae genera due to the concerns about concerted evolution and paralogy

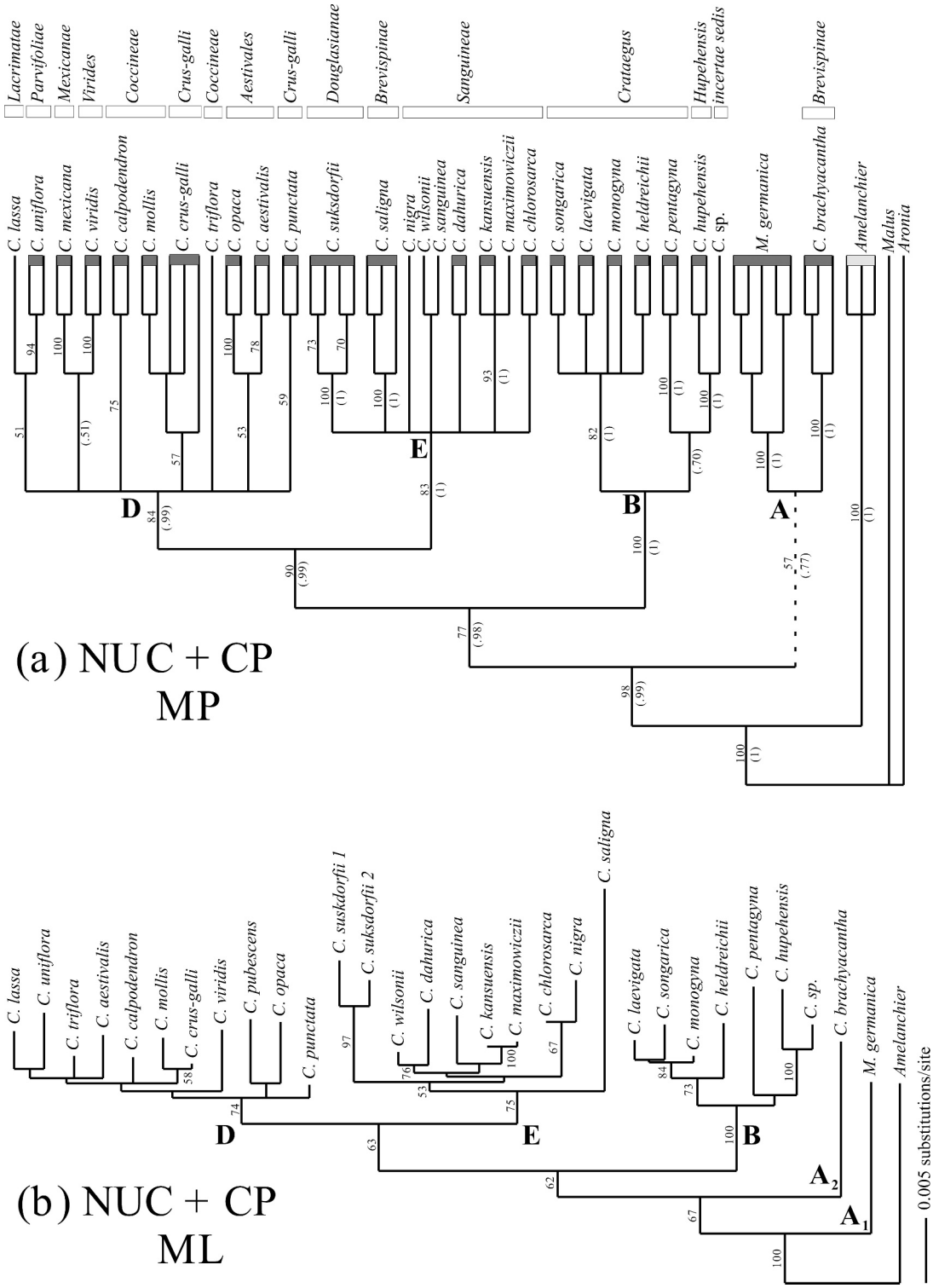

FIG. 5. Trees based on combined nuclear and chloroplast data generated by (a) maximum parsimony (MP) and (b) maximum likelihood (ML), using the TVM model with lnL: −11,001.39, I = 0.51 and G = 0.57. In (a), bootstrap (BS; above branch) and posterior probability (BI; below branch) values > 50% are indicated; the dotted line represents the branch that collapses in the maximum likelihood analysis. In (b), nodes with bootstrap (BS) values > 50% are indicated above branch. *Crataegus marshallii*, *C. phaenopyrum*, *C. spathulata*, and *Mespilus canescens* were omitted from the analyses because of their conflicting positions in the nuclear (Fig. 4a) and chloroplast trees (Fig. 4b).

especially in hybrid and polyploid taxa (Bailey et al. 2003). Genes such as *waxy* and *s6pdh* have been recently shown by Southern hybridization to contain more than one copy in Rosaceae (Evans et al. 2000; Bortiri et al. 2002). *LEAFY*, a floral homeotic gene of the MADS box gene family that controls meristem development in *Arabidopsis* (Blazquez et al. 1997), is suggested to be single copy through the loss of its paralogous copy during angiosperm evolution (Frohlich and Meyerowitz 1997). Although more than one ortholog is present in species of *Malus* (Wada et al. 2002), our PCR products appear to be single bands and introns have been shown to be phylogenetically informative not only here but also in other Rosaceae, e.g. *Neillia* and *Stephanandra* (Oh and Potter 2003, 2005). Variability of the *LEAFY* sequences in *Crataegus* and *Mespilus* was comparable to that in the ITS region (Table 1), but substantial divergence (three times more than in ITS) was found between the ingroup and *Malus* and *Aronia*. Such divergence, on one hand, demonstrates the potential utility of using *LEAFY* elsewhere in Pyreae. However, as shown in this study, when the sequences being analyzed are too divergent, or when rates of evolution show considerable variation among sequences, a spurious phylogeny could be produced due to long-branch attraction (Felsenstein 1978). One approach to minimize this effect is to include sequences with more changes along short internal branches in order to reduce the differences in branch length. An alternative is to include more samples to break down long branches of the diverged outgroup taxa. In our case, we took the former approach and combined the ITS and *LEAFY* data to obtain a more accurate phylogeny.

Phylogenetic Utility of Chloroplast Regions in Pyreae. Regions such as *rbcL, matK*, and *trnL-F* have been used in earlier studies of Rosaceae phylogeny (Chase et al. 1993; Morgan et al. 1994; Potter et al. 2002). Some of these were shown to be informative at the generic level within subfamilies such as Amygdaloideae (Lee and Wen 2001; Bortiri et al. 2002) and Rosoideae (Eriksson et al. 2003). Other regions such as *rpl16, rps16, trnL, ndhF*, and *rbcL-atpB* have been used singly or together to infer intergeneric relationships within Pyreae (Campbell et al., in press), but the resolution was not as high as a single nuclear ITS region. In this regard, the attempt to reconstruct a maternal phylogeny of genera in the Pyreae, especially at lower taxonomic levels, was considered challenging. Nevertheless, chloroplast regions vary broadly in their evolutionary rates that give different amounts of phylogenetic signal at any given taxonomic level (Zurawski and Clegg 1987; Golenberg et al. 1993; Olmstead and Palmer 1994). In this study, we resolved a species-level phylogeny of *Crataegus* and *Mespilus* using four intergenic regions of the chloroplast genome that have never been used in the Rosaceae, *trnG-trnS, psbA-trnH, trnH-rpl2*, and *rpl20-rps12*. The combined data yielded as much as 5.84% polymorphism (Table 1) among all taxa examined and gave a topology compatible with the nuclear results.

Implications of Nuclear and Chloroplast Data Incongruence. The combined nuclear trees (Figs. 2, 4a) were considered to be good hypotheses for evolutionary relationships in *Crataegus* and *Mespilus* because of the high degree of congruence between parsimony, maximum likelihood, and Bayesian analyses, in addition to the higher resolution, bootstrap, and posterior probability values obtained from the combined datasets. However, comparison with the chloroplast results (Figs. 3, 4b) demonstrates conflicts such as the placement of three eastern North American taxa *C. marshallii, C. phaenopyrum*, and *C. spathulata*. These conflicts are important and will be discussed in more detail elsewhere, as part of our overall appraisal of relationships within *Crataegus*.

Of greater concern here is the placement of *C. brachyacantha* and the *Mespilus* species. *Crataegus brachyacantha* occurs naturally in Louisiana, eastern Texas, and adjacent portions of Arkansas and Oklahoma; there is also a single record for southwestern Georgia (Phipps 1998). It is noteworthy for its secondary leaf venation (Table 2), its petals that may turn orange upon drying, and for its dark purple to black fruit, covered with a waxy bloom. Its relatively isolated position within the genus is best accommodated by transferring *C. saligna*, until now the only other member of section *Brevispinae*, out of that section and into section *Douglasianae*. This transfer is the best solution for *C. saligna* at this time, pending more comprehensive analyses of sections *Douglasianae* and *Sanguineae* (Figs. 1–5).

One cause of the incongruence between the nuclear and chloroplast trees could be the recent occurrence of hybridization between early-diverged taxa. *Mespilus canescens* and *M. germanica* share a common ancestor, as shown by the nuclear data (Figs. 2, 4a), but *M. canescens* is shown to be more closely related to *C. brachyacantha* than it is to *M. germanica* based on the chloroplast data (Figs. 3, 4b; cf. Verbylaitė et al. 2006). Conflicting topologies like these suggest a hybrid origin of *M. canescens*, with *C. brachyacantha* as the probable maternal parent with over 99% identity in the chloroplast sequences.

Hybridization between *C. brachyacantha* and *M. germanica* could have occurred if the latter was

TABLE 2. Morphological variation, ploidy level, and geographic distribution (Characters 1–11, Appendix 2) as they are expressed in *Amelanchier*, *Mespilus*, and exemplar species of *Crataegus* spp. (Appendix 1 and Figs. 1–4). In bold, character states that apply to the genus as a whole. NA, character not applicable; ND, no data. Data from field observations and herbarium specimens, Robertson et al. (1992), Phipps et al. (2003). [1]The secondary venation of this taxon is inadequately described by the term camptodromous because secondary veins frequently lead to the margin, but form nodes just below the sinuses between the marginal crenations.

Characters	1	2	3	4	5	6	7	8	9	10	11
OUTGROUPS											
Amelanchier											
A. arborea	**1**	**0**	**0**	**NA**	0	1	1	0(1/2)	4	0	1
A. bartramiana	**1**	**0**	**0**	**NA**	0	1	1	0	4	0	1
Aronia											
A. arbutifolia	**0**	**0**	**0**	**NA**	0	2	1	0/1/2/3	1	0/2	1
Malus											
M. angustifolia	**0**	**0**	**0**	**NA**	0	0	1	0	2	ND	1
INGROUPS											
Mespilus											
M. germanica	**0**	**1**	**1**	0	1	1/2	0	0	0	0	3
M. canescens	**0**	**1**	**1**	0	0	0/1/2	1	0	1	1	1
Crataegus											
C. brachyacantha	**0**	**1**	**1**	**1**	0	2[1]	1	0	4	0	0
CLADE B											
C. monogyna	**0**	**1**	**1**	**1**	0	0	1	4	1	0(1)	3
C. pentagyna	**0**	**1**	**1**	**1**	0	0	1	0	4	0	3
C. pinnatifida	**0**	**1**	**1**	**1**	0	1	1	0(1/2)	1	0(1/2/3)	2
CLADE D											
C. calpodendron	**0**	**1**	**1**	**1**	0	0	1	1/2	1	0(2/3)	1
C. crus-galli	**0**	**1**	**1**	**1**	0	0/1	2(1)	(0/1)/2/3/4	1	(0/1)/2	1
C. opaca	**0**	**1**	**1**	**1**	0	0	1	0/1/2	1	0	1
C. mexicana	**0**	**1**	**1**	**1**	0	0	1	0	2	0	1
C. phaenopyrum	**0**	**1**	**1**	**1**	0	0/1	1	0/1/2	1	1/2	1
C. triflora	**0**	**1**	**1**	**1**	0	0	0	0/1/2	1	0/1/2	1
C. uniflora	**0**	**1**	**1**	**1**	1	0/1	1	0/1/2	1(2)	1	1
C. viridis	**0**	**1**	**1**	**1**	0	0/1	1	0	1	0/1	1
CLADE E											
C. chlorosarca	**0**	**1**	**1**	**1**	0	0	1	0	4	0	2
C. nigra	**0**	**1**	**1**	**1**	0	0	1	0/1	4	0	3
C. saligna	**0**	**1**	**1**	**1**	0	1	1	0	4	0	0
C. suksdorfii sensu lato	**0**	**1**	**1**	**1**	0	0	1	0(1/2)	4	0/1/2	0
C. sanguinea	**0**	**1**	**1**	**1**	0	0	1	(0/1)2	1	0(1/2)	2
C. wilsonii	**0**	**1**	**1**	**1**	0	0	1	2/3	1	0	2

cultivated within the range of *C. brachyacantha* sometime in the past 150–200 years. In fact, Baird and Thieret (1989) refer to an 1893 report of cultivation of *M. germanica* at an agricultural station in Louisiana, suggesting that there is no reason to exclude this possibility. Hybridization among *Crataegus* species is well-documented (e.g. Christensen 1992; Phipps 2005), although its frequency and significance is debated. The factors likely most relevant to whether hybridization between *M. germanica* and *C. brachyacantha* could have occurred include proximity and phenology (Campbell et al. 1991). Hawthorns and medlars have relatively unspecialized entomophilous flowers with abundant pollen and are apparently pollinated primarily by bees (Dickinson 1985; Dickinson et al. 1996). Although the number of flowers per inflorescence varies (Table 2), even in many-flowered inflorescences anthesis is usually completed within a week or less, at a time that appears to be highly species-specific and controlled by vernal accumulated heat (Dickinson and Phipps 1986; Smith and Phipps 1988). Nothing, however, is known about the relative timing of anthesis in *M. germanica* and *C. brachyacantha*.

A single population of red-fruited *M. canescens* was discovered in 1970 in Konecny Grove, a small nature reserve in Arkansas, and this site remains the only one at which this species is known to occur naturally (Phipps 1990). Trees of *M. canescens* are triploid, whereas individuals of *M. germanica* that have been studied are exclusively diploid (Appendix 1; Talent and Dickinson 2005). A possible origin of *M. canescens* from a cross

between two Pyreae species was considered by Phipps but dismissed ''due to the lack of at least two suitable candidates'' (Phipps 1990). Nevertheless, petals of *M. canescens* resemble those of *C. brachyacantha* in turning a faint orange color upon drying and, in the analysis of 44 isozyme phenotypes for eight enzyme systems (Phipps et al. 1991), the two *Mespilus* species for the most part exhibited a subset of the 35 phenotypes found in 21 *Crataegus* species. Two phenotypes were unique to *M. canescens*, and two more were also found in *M. germanica* but not in any of the *Crataegus* species. *Mespilus canescens* shared two phenotypes with *C. brachyacantha*, and three with *C. chlorosarca*. Both sexual and graft hybrids between *M. germanica* and *Crataegus* species are known, and have been described as the nothogenera ×*Cratae-mespilus* E. G. Camus and +*Crataego-mespilus* Simon-Louis ex Bellair, respectively (Byatt et al. 1977; Baird and Thieret 1989). The *Crataegus* parents of the sexual hybrids, ×*C. grandiflora* (Smith) E. G. Camus and ×*C. gillotii* Beck, are inferred to be, respectively, *C. laevigata* and *C. monogyna*. In the diploid ×*C. grandiflora*, pollen meiosis is disturbed, and pollen viability is around 5%, in contrast with viability in excess of 95% for all three parental diploids (Byatt et al. 1977). These results are more extreme than those from studies of hybridization between introduced *C. monogyna* and North American diploid *Crataegus* species (Love & Feigen 1978; Wells & Phipps 1989) in which the pollen stainability of putative hybrids was typically greater than 40% (pollen stainability of the parental species was 80–95%).

A scenario that would account for the known facts can be outlined as follows. At some time, probably in the nineteenth century, pollen from cultivated *M. germanica* was transferred to stigmas of *C. brachyacantha*, resulting in hybrid seed formation. Hybrid individuals grew to maturity but were infertile, due to irregular meiosis as in ×*C. grandiflora*. Under these circumstances only occasional seeds were set, and these resulted from the fertilization of unreduced female gametes of the primary hybrid by reduced male gametes from the pollen of either *M. germanica* or a native, diploid (and probably red-fruited) *Crataegus* species. Such a scenario is at least as plausible as an origin for *M. canescens* as an autotriploid from a now extinct species of *Mespilus* that persisted in North America since the divergence of *Mespilus* and *Crataegus*. Recognition of what has up to now been known as *M. canescens* as a nothospecies of ×*Crataemespilus* thus would seem warranted on the basis of the molecular results obtained here if it were not for the question, discussed below, of whether to maintain *Mespilus* as a genus distinct from *Crataegus*.

Re-evaluation of Generic Limits. After removing conflicts due to hybridization or other factors, the analyses of the combined nuclear and chloroplast sequence data (Fig. 5) suggest that *C. brachyacantha* is sister to the remaining *Crataegus* species rather than to *M. germanica*. This raises the question of whether *M. germanica* should be included within *Crataegus*, since there appear to be fewer differences between these two genera than between them and their sister genus, *Amelanchier* (Table 2). For the characters that were suggested earlier as distinguishing *Mespilus* (hence *M. canescens*) from *Crataegus* (Table 2, Appendix 2), a closer examination suggests that these two genera are more similar than has been acknowledged previously (Table 2).

Differences between the *Mespilus-Crataegus* clade and *Amelanchier* include the timing of replacement growth on fertile short shoots, disposition of the ovules within the locule, and composition of the mature fruit and seeds (Aldasoro et al. 2005; Table 2, Appendix 2). Some of the characters that might provide synapomorphies for *Crataegus* (relative to *Mespilus*; Phipps 1990) in fact vary within *Crataegus*, such as short shoot leaf margination, shape, and venation pattern, the numbers of flowers per inflorescence, and number of stamens per flower (Table 2). Only whether the petals are notched apically (emarginate) and the way in which the apices of the pyrenes are, or are not, exposed in the fruit really distinguish the two species currently ascribed to *Mespilus* from *Crataegus*.

Although only about a quarter or fewer of species in *Crataegus* are included in our sample, out of the 15 sections in the genus we have covered all but section *Cuneatae* Rehder ex Schneider (eastern Asia), and have at least two individuals from different localities for most species (Appendix 1). The SH test of the combined nuclear and chloroplast data did not reject the hypothesis of *Crataegus* and *Mespilus* being two distinct lineages, but only when *M. canescens* was removed from the dataset. Since a hybrid origin of *M. canescens* is plausible and justifies its removal from the phylogenetic analyses, *Crataegus* and *Mespilus* can still be treated as two distinct genera. However, because the number of morphological differences supporting the branch between the *Mespilus-Crataegus* clade and *Amelanchier* is considerably greater than those distinguishing *Mespilus* and *Crataegus* from each other, it seems more reasonable to sink the smaller genus in the larger one and create a new section to accommodate it. Accord-

ingly, we make the following new combinations, and a new nothosection to accommodate one of them.

Crataegus Linnaeus sect. ***Mespilus*** T. A. Dickinson & E. Y. Y. Lo stat. nov., comb. nov. (*Mespilus* Linnaeus in Sp. Pl. 1: 478, 1753; Gen. Pl., ed. 5: 549, 1754).

Ab omnibus sectionibus alteris Crataegi differt fructibus apicibus pyrenarum omnino tectis, necnon coniunctione foliorum venatione semicraspedodroma, non lobatorum, inflorescentiarum 1(–2)-florarum, atque staminum 30(–40) in quoque flore.

Deciduous trees or shrubs to 10 m, deciduous. Bark gray-brown on young branches, becoming gray with age. Shoots dimorphic, lateral short shoots sympodial, sometimes developing as aphyllous thorns especially in wild genotypes; borne on twigs 2 years or more old, each bearing 5–7 or more preformed leaves, and often inflorescences; long shoots with both preformed and neoformed leaves. Leaves alternate, spirally arranged, simple, 5–10(–15) cm long, 3–5 cm wide; stipules deciduous, distinct; petioles present. Leaf blade pinnately veined, secondary venation semi-craspedodromous. Leaf blades elliptic, apex pointed. Inflorescences terminal on short shoots, comprising 1(–2) flowers. Flowers 3–5 cm across when open, bisexual, pentamerous, epigynous; sepals 5; petals 5, sometimes emarginate apically; stamens 30 (–40), pistil 1, ovary inferior, (4–)5-locular; placentation axile; ovules 2 per locule, anatropous, apitropic, initiated collaterally at base of locule but becoming superposed, with a single funicular obturator adjacent the micropyle of the lower ovule; styles (4–)5, stigmas wet-papillate. Fruits polypyrenous drupes (''pomes''), brown at maturity, 1.5–3 cm in diameter (–7 cm in cultivated genotypes), completely enclosing five 1-seeded pyrenes, the free portion of the hypanthium forming a low wall around the disk almost as wide as the fruit itself, the calyx lobes typically erect; seed coat membranous; endosperm present, thin at maturity; embryo straight, as long as seed; cotyledons flat. One species, *C. germanica* (L.) K.Koch.

Crataegus nothosection ***Phippsara*** T. A. Dickinson & E. Y. Y. Lo, nothosect. nov. (*Crataegus* sect. *Mespilus* × *Crataegus* sect. *Brevispinae* Beadle ex C.K.Schneid. × unknown section)

Deciduous trees or shrubs to 7 m. Bark gray-brown on young branches, flaking with age on the trunk. Shoots dimorphic, lateral short shoots sympodial, occasionally developing as aphyllous thorns; borne on twigs 2 years or more old, each bearing 5–7 or more preformed leaves, and often inflorescences; long shoots with both preformed and neoformed leaves. Leaves alternate, spirally arranged, simple, 3–5 cm long, (1–)1.5–2 cm wide, canescent; stipules deciduous, distinct; petioles present. Leaf blade pinnately veined, secondary venation semi-craspedodromous. Leaf blades elliptic, apex pointed. Inflorescences flat-topped panicles usually terminating short shoots, pubescent, and comprising (2–)5–10 flowers. Flowers 1.5–2 cm across when open, bisexual, pentamerous, epigynous; sepals 5; petals 5, emarginate apically, acquiring an orange tinge upon drying; stamens 20, pistil 1, ovary inferior, (4–)5-locular; placentation axile; ovules 2 per locule, superposed; styles (4–)5. Fruits polypyrenous drupes (''pomes''), red at maturity, 1–1.5 cm in diameter, completely enclosing five 1-seeded pyrenes. One species, *C.* ×*canescens* (J.B.Phipps) T.A.Dickinson & E.Y.Y.Lo. Etymology: James B. Phipps is the preeminent North American student of hawthorns and medlars. His energetic fieldwork and detailed revisionary studies have provided a wealth of new information about these plants as they occur across the continent.

Crataegus ×***canescens*** (J. B. Phipps) T. A. Dickinson & E. Y. Y. Lo comb. nov. – *Mespilus canescens* J.B.Phipps, Syst. Bot. 15: 26–32. Lawrence, Kansas 1990.

Crataegus nothosection ***Cratae-mespilus*** (E. G. Camus) T. A. Dickinson & E. Y. Y. Lo stat. nov., comb. nov. – ×*Cratae-mespilus* E.G.Camus, Journal de Botanique 13: 326, Paris 1899.

Crataegus ×***gillotii*** (Beck) T. A. Dickinson & E. Y. Y. Lo comb. nov. – ×*Cratae-mespilus gillotii* Beck, Icones Florae Germanicae et Helveticae 25: 30, t. 107, Leipzig, Gera 1914.

The combination *Crataegus* ×*grandiflora* K.Koch has already been made and, under Article 4 of the International Code of Nomenclature for Cultivated Plants (Brickell et al. 2004), scientific names for graft-chimaeras below the rank of genus are unnecessary (they may be named as cultivars). Because the transfer of *Crataegus* species to *Mespilus* has already been made (Scopoli 1772), we have elsewhere (Talent et al. submitted) proposed conservation of *Crataegus* over *Mespilus* in the interest of nomenclatural stability. There are potentially hundreds of new combinations that would be required if the phylogenetic results obtained here are to inform taxonomy and *Crataegus* is not conserved over *Mespilus*.

To conclude, molecular and morphological data indicate no clear genetic distinction between

Crataegus and *Mespilus*. Although there is a certain arbitrariness in the assignment of taxonomic rank, we believe that the taxonomic solution that best reflects both the molecular phylogeny and the morphological data, as well as causing minimum disruption of existing nomenclature, is to sink the genus *Mespilus* in *Crataegus* as a new, monotypic section. *Mespilus canescens* is readily accommodated as an intersectional hybrid named as a nothospecies in a new nothosection ×*Phippsara*. Together with a monotypic section *Brevispinae*, these realignments combine a phylogenetic basis for the classification of hawthorns and medlars with the greatest nomenclatural stability.

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APPENDIX 1. Locality and vouchers data for outgroup, *Mespilus*, and *Crataegus* taxa used for molecular analyses. Nomenclature follows that used by Talent and Dickinson (2005). Except as noted, locality data are state (or Canadian provinces) and county (or parish) where leaf samples and herbarium vouchers were collected. All vouchers were deposited in the Green Plant Herbarium of the Royal Ontario Museum (TRT) unless noted otherwise; except as noted vouchers were collected by TAD (vouchers for all cultivated material is TAD s. n. at TRT unless indicated otherwise). Ploidy data as reported in Talent and Dickinson (2005); in bold, ploidy determinations from that paper based on the accession studied here. [1]Campbell et al. (1987), vouchers in MAINE; [2]A. A. Dönmez coll. (vouchers in HUB); [3]det. R. Lance; [4]N. Talent coll.; [5]D. Kandalepas coll.

Genus, Section, and Series	Species	Voucher	Source	Ploidy level ($x = 17$)
Amelanchier Medik.	*A. arborea* (Michx. f.) Fernald	2003-1	Alabama, DeKalb	**2*x***
	A.bartramiana (Tausch) Roemer	B5[1]	Maine, Somerset	2*x*
		B9[1]		
Aronia Mitch.	*Aronia arbutifolia* (L.) Ell.	2003-2	Alabama, DeKalb	**ca. 4*x***
Malus Miller	*M. angustifolia* (Aiton) Michx.	2003-3		2x, 3x
Mespilus L.				
	M. canescens Phipps	2003-35-03	Arkansas, Prairie	**3*x***
		2003-36-11		**3*x***
		2003-37-13		**3*x***
		2003-38-17		**3*x***
		2003-39-18		**3*x***
		2003-40-19		**3*x***
		2003-41-20		**3*x***
		2003-42-22		**3*x***
		2003-43-24		**3*x***
	M. germanica L.	W. Hess & M. Linden 6216V93 (cult.)	M645-80/32-28, Morton Arboretum, Chicago, Illinois	**2*x***
		W. Hess & M. Linden 6216V93 (cult.)	M682-80/50-62	**2*x***
		S. M. Bailleul s.n. (cult.)	113-48-56, Jardin Botanique de Montréal, Montréal, Québec	**2-3*x***
		UCBG78.0184 (cult.)	University of California Botanical Garden, Berkeley, California	
		AA727-89B	Arnold Arboretum, Boston, Massachusetts	
		AAD11457[2]	Turkey, Artvin	
		AAD11600[2]	Turkey, Kirkklareli	
		AAD11619[2]	Turkey, Istanbul	
		AAD11656[2]	Turkey, Bursa	
		AAD11660[2]	Turkey, Bolu	
		AAD11687[2]		
Crataegus L.				
Mexicanae Loud.				
Mexicanae (Loud.) Rehder	*C. mexicana*	UCBG76-2049 (cult.)	University of California Botanical Garden, Berkeley, California	2*x*
Parvifoliae Loud.				
Parvifoliae (Loud.) Rehder	*C. uniflora* Münchh.	2003-26	Alabama, Autauga	**3*x***
		2003-52	Virginia, Franklin	**3-4*x***

APPENDIX 1. Continued.

Genus, Section, and Series	Species	Voucher	Source	Ploidy level ($x = 17$)
Crataegus				
Crataegus	*C. laevigata* Poir.	Zika 18472	Washington, San Juan	**2*x***
	C. laevigata Poir.	Zika 18473		**4*x***
	C. *monogyna* Jacq.	Love C-2003-25	Oregon, Lane	**3*x***
	C. *monogyna* Jacq.	99FW7-11	Oregon, Linn	2*x*
	C. songarica K. Koch	AA198-65A (cult.)	Arnold Arboretum, Boston, Massachusetts	**4*x***
	C. songarica K. Koch	AA113-96A (cult.)		
Apiifoliae (Loudon) Rehder	*C. marshallii* Egglest.	2003-05	Alabama, Dekalb	**2-3*x***
	C. marshallii Egglest.	2003-30	Mississippi, Scott	**2-3*x***
Orientales (C.K.Schneid.) Pojark.	*C. heldreichii* Boiss.	AA238-71A (cult.)	Arnold Arboretum, Boston, Massachusetts	**2*x***
Pentagynae (C.K.Schneid.) Russanov	C. *pentagyna* Waldst. & Kit.	AA94-85B (cult.)	Arnold Arboretum, Boston, Massachusetts	2*x*
		Christensen 312 (cult.)	Denmark, Taastrup	
Sanguineae Zabel ex Schneider				
Nigrae (Loudon) Russanov	*C. chloroscara* Maxim.	AA281-71A (cult.)	Arnold Arboretum, Boston, Massachusetts	**2*x***
	C. kansuensis Wilson	2002-02A (cult.)	AA-EN101, Arnold Arboretum, Boston, Massachusetts	2*x*
		AA12-95 (cult.)		
	C. maximowicizii Schneider	2002-04A (cult.)	AA309-97, Arnold Arboretum, Boston, Massachusetts	2*x*, 3*x*, 4*x*
	C. nigra Waldst. and Kit.	Christensen 294 (cult.)	Denmark, Taastrup	2*x*
Sanguineae (Zabel ex Schneider) Rehder	*C. dahurica* Koehne ex Schneider	AA71-73A (cult.)	Arnold Arboretum, Boston, Massachusetts	**2*x***
		AA-EN250-2000 (cult.)		
	C. sanguinea Pall. ex Bieb.	JBM1232-49 (cult.)	Jardin Botanique de Montréal, Montréal, Québec	2*x*, 3*x*, 4*x*
	C. wilsonii Sarg.	AA271-84A (cult.)	Arnold Arboretum, Boston, Massachusetts	**2*x***
		AA749-74A (cult.)		**2*x***
Hupehensis J.B.Phipps.				
Hupehenses J.B.Phipps.	*C. hupehensis* Sarg.	AA356-81B (cult.)	Arnold Arboretum, Boston, Massachusetts	**2-3*x***
		AA356-81C (cult.)		
Cordatae Beadle ex Egglest.				
Cordatae (Beadle ex Egglest.) Rehder	*C. phaenopyrum* (L. f.) Medikus	99ME1 (cult.)	Maine, Penobscot	3*x*, 4*x*
		AA195-52B (cult.)	Arnold Arboretum, Boston, Massachusetts	
Virides (Beadle ex Sarg.) Schneider				
Virides (Beadle ex Sarg.) Rehder	*C. viridis* L.	2003-44	Arkansas, Prairie	**2*x*+**
		2003-45		**2-3*x***
Microcarpae Loud.				
Microcarpae (Loud.) Rehder	*C. spathulata* Michx.	2003-6	Georgia, Floyd	**2-3*x***
		2003-34	Louisiana, Boissier	**2-3*x***
Lacrimatae (J.B.Phipps) J.B.Phipps				
Lacrimatae J.B.Phipps	*C. lassa* Beadle	2003-18[4]	Alabama, Dallas	**high**
Aestivales (Sarg.) Schneider				
Aestivales (Sarg.) Rehder	*C. aestivalis* (Walt.) T. &G.	NC1992-250 (cult.)[4]	North Carolina Arboretum, Asheville, North Carolina	**low**

APPENDIX 1. Continued.

Genus, Section, and Series	Species	Voucher	Source	Ploidy level ($x = 17$)
		Talent 321[3]	North Carolina, Buncombe	**low**
	C. opaca Hook. & Arn.	2003-33 (cult.)	Louisiana, Sabine	**2*x***
		AA387-96A (cult.)	Arnold Arboretum, Boston, Massachusetts	
		2001-1[5]	Texas, Jasper	
Brevispinae Beadle ex Schneider				
Brevispinae (Beadle ex Schneider) Rehder	*C. brachyacantha* Sarg. & Engelm.	2000-11	Texas, Jasper	
		2001-3A[5]	Texas, Jasper	
		2003-32	Louisiana, Sabine	**2-3*x***
		Reid 5203	Louisiana, Morehouse	**2-3*x***
	C. saligna Greene	99FW1/1	Colorado, Gunnison	2*x*, 2-3*x*
		2001-4A	Colorado, Rio Blanco	
		2001-7A		
Douglasianae Loud.				
Douglasianae (Loud.) Poletiko	*C. suksdorfii* (Sarg.) Kruschke	D1619A	Montana, Powell	4x
		2001-27A	Montana, Lake	
		Love C-2003-11	Oregon, Lane	2-3x
		Zika 18477	Oregon, Columbia	**2*x***
		Zika 18483	Oregon, Washington	
		Zika 18485	Washington, Clark	
		99FW8/9	Washington, Klickitat	
		99FW8/12		
Crus-galli Loud.				
Crus-galli (Loud.) Rehder	*C. crus-galli* L.	Talent 213A	Alabama, Montgomery	**2*x***
		Talent 286	Georgia, Houston	low
		2003-15	Alabama, Lowndes	4*x*
	C. punctata Jacq.	2000-26	Ontario, Lambton	
		BB4	Ontario, Bruce	**2*x***
Coccineae Loud.				
	C. sp.	2003-4	Alabama, DeKalb	
Macracanthae (Loud.) Rehder	*C. calpodendron* (Ehrh.) Medikus	Talent 166	Ontario, Middlesex	**2*x***
		Talent 172	Ontario, Niagara	**2*x***
		2000-28	Ontario, Middlesex	
		AA277-68A (cult.)	Arnold Arboretum, Boston, Massachusetts	
Molles (Beadle ex Schneider) Rehder	*C. mollis* (T. and G.) Scheele	D1655	Ontario, Middlesex	**2-3x**
		Talent 208 (cult.)	Wisconsin, Madison	**2*x***
Triflorae (Beadle) Rehder	*C. triflora* Chapm.	Talent 290a[3]	Georgia, Floyd	**2*x***
incertae sedis	*C.* sp.	RBG 54705	Royal Botanic Garden, Hamilton	**2*x***

APPENDIX 2. Morphological characters and their states, together with ploidy level and geographic distribution, as they are expressed in *Amelanchier*, *Mespilus*, and *Crataegus* species.

1. Sympodial replacement growth on reproductive short shoots is **proleptic (0;** during the following growing season) or **sylleptic (1;** during the same growing season as flowering), from an axillary bud below the terminal inflorescence. **2.** Disposition of ovules within the locule at anthesis is typically **collateral (0)** or the ovules are **superposed (1)** (Decaisne 1874; Evans and Dickinson 2005). **3.** Seeds are enclosed **in a cartilaginous core (0)** or **within a woody endocarp, or pyrene (1)** (Rohrer et al. 1991). **4.** In the mature fruit the apices of the pyrenes **are covered by epidermis (0)** or **are exposed (1)** (Decaisne 1874; Koehne 1890). **5.** Inflorescence typically **multiflorous (0)** or **uniflorous (1)** (Rohrer, Robertson and Phipps 1994). **6.** Secondary venation of short shoot leaves typically **craspedodromous (0)** (Robertson et al. 1992, Fig. 1; Leaf Architecture Working Group 1999, Fig. 29.7), **semi-craspedodromous (1)** (Leaf Architecture Working Group 1999, Fig. 29.8), or (eu-) **camptodromous (2)** (Robertson et al. 1992, Fig. 2; Leaf Architecture Working Group 1999, Fig. 29.3). **7.** Flowers, mean number of stamens $>$ **25 (0)**, **15–25 (1)**, or $<$ **15 (2)**. **8.** Flowers, number of gynoecial units (locules, styles) $\geq$ **5 (0)**, **4 (1)**, **3 (2)**, **2 (3)**, or **d 1 (4)**. **9.** Fruits **brown (0)**, **red (1)**, **yellow (2)**, **white (3)**, or **blue, purple, or black (4)**. **10.** Ploidy level $2n = 2x =$ **34 (0)**, $2n = 3x =$ **51 (1)**, $2n = 4x =$ **68 (2)**, or $2n = 5x =$ **85 (3)**. **11.** Geographic distribution **western North America (0)**, **eastern North America (1)**, **eastern Eurasia (2)**, or **western Eurasia (including northern Africa) (3)**.

APPENDIX 3. GenBank accession numbers of representative species used in the phylogenetic reconstruction here. Abbreviations for voucher material follow Appendix 1; GenBank numbers are in the order *trnS-trnG, psbA-trnH, trnH-rpl2, rpl20-rps12*, ITS, *leafy*.

Amelanchier arborea (Michx. f.) Fernald *2003-1* EF127115, EF127152, EF127189, EF127226, EF127041, EF127078. ***Aronia arbutifolia*** (L.) Ell. *2003-2* EF127117, EF127154, EF127191, EF127228, EF127043, EF127080. ***Malus angustifolia*** (Aiton) Michx. *2003-3* EF127116, EF127153, EF127190, EF127227, EF127042, EF127079. ***Mespilus canescens*** Phipps *2003*-37-13 EF127099, EF127136, EF127173, EF127210, EF127039, EF127076. ***M. germanica*** L. *W. Hess & M. Linden 216v93* (cult.) EF127098, EF127135, EF127172, EF127209, EF127040, EF127077. ***Crataegus mexicana*** UCBG76-2049 (cult.) EF127082, EF127119, EF127156, EF127193, EF127021, EF127058. ***C. uniflora*** Münchh. *2003-26* EF127112, EF127149, EF127186, EF127223, EF127020, EF127057. ***C. laevigata*** Poir. *Zika 18472* EF127093, EF127130, EF127167, EF127204, EF127015, EF127052. **C. *monogyna*** Jacq. *99FW7-11* EF127091, EF127128, EF127165, EF127202, EF127014, EF127051. ***C. songarica*** K. Koch AA198-65A (cult.) EF127092, EF127129, EF127166, EF127203, EF127036, EF127073. ***C. marshallii*** Egglest. *2003-05* EF127095, EF127132, EF127169, EF127206, EF127037, EF127074. ***C. heldreichii*** Boiss. AA238-71A (cult.) EF127090, EF127127, EF127164, EF127201, EF127016, EF127053. **C. *pentagyna*** Waldst. & Kit. AA94-85B (cult.) EF127094, EF127131, EF127168, EF127205, EF127035, EF127072. ***C. chloroscara*** Maxim. AA281-71A (cult.) EF127110, EF127147, EF127184, EF127221, EF127009, EF127046. ***C. kansuensis*** Wilson AA12-95 (cult.) EF127108, EF127145, EF127182, EF127219, EF127029, EF127066. ***C. maximowicizii*** Schneider *2002-04A* (cult.) EF127109, EF127146, EF127183, EF127220, EF127030, EF127067. ***C. nigra*** Waldst. and Kit. *Christensen 294* (cult.) EF127107, EF127144, EF127181, EF127218, EF127007, EF127044. ***C. dahurica*** Koehne ex Schneider AA-EN250-2000 (cult.) EF127105, EF127142, EF127179, EF127216, EF127028, EF127065. ***C. sanguinea*** Pall. ex Bieb. JBM1232-49 (cult.) EF127106, EF127143, EF127180, EF127217, EF127027, EF127064. ***C. wilsonii*** Sarg. AA749-74A (cult.) EF127104, EF127141, EF127178, EF127215, EF127008, EF127045. ***C. hupehensis*** Sarg. AA356-81B (cult.) EF127111, EF127148, EF127185, EF127222, EF127038, EF127075. ***C. phaenopyrum*** (L. f.) Medikus 99ME1 (cult.) EF127096, EF127133, EF127170, EF127207, EF127034, EF127071. ***C. viridis*** L. *2003-45* EF127113, EF127150, EF127187, EF127224, EF127013, EF127050. ***C. spathulata*** Michx. *2003-34* EF127097, EF127134, EF127171, EF127208, EF127033, EF127070. ***C. lassa*** Beadle *2003-18*[4] EF127081, EF127118, EF127155, EF127192, EF127024, EF127061. ***C. aestivalis*** (Walt.) T. &G. *Talent 321*[3] EF127089, EF127126, EF127163, EF127200, EF127023, EF127060. ***C. opaca*** Hook. & Arn. *2003-33* (cult.) EF127088, EF127125, EF127162, EF127199, EF127022, EF127059. ***C. brachyacantha*** Sarg. & Engelm. *2000-11* EF127100, EF127137, EF127174, EF127211, EF127032, EF127069. ***C. saligna*** Greene 99FW1/1 EF127101, EF127138, EF127175, EF127212, EF127031, EF127068. ***C. suksdorfii*** (Sarg.) Kruschke *Love C-2003-11* EF127103, EF127140, EF127177, EF127214, EF127025, EF127062. *Zika 18477* EF127102, EF127139, EF127176, EF127213, EF127026, EF127063. ***C. crus-galli*** L. *Talent 213A* EF127087, EF127124, EF127161, EF127198, EF127010, EF127047. ***C. punctata*** Jacq. *BB4* EF127086, EF127123, EF127160, EF127197, EF127011, EF127048. ***C. calpodendron*** (Ehrh.) Medikus *Talent 172* EF127083, EF127120, EF127157, EF127194, EF127018, EF127055. ***C. mollis*** (T. and G.) Scheele *D1655* EF127085, EF127122, EF127159, EF127196, EF127012, EF127049. ***C. triflora*** Chapm. *Talent 290a*[3] EF127084, EF127121, EF127158, EF127195, EF127019, EF127056. ***C***. sp. RBG 54705 EF127114, EF127151, EF127188, EF127225, EF127017, EF127054.